IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the patent application of David Honc

Serial No. 10/632,095 Group Art Unit 1632

Filed 08/01/2003 Examiner: Licto

For: ***"DNA VACCINES THAT EXPRESS MUTANT ADP-RIBOSYLTRANSFERASE TOXINS WHICH DISPLAY REDUCED, OR ARE DEVOID OR, ADP-RIBOSYLTRANSFERASE ACTIVITY"***

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I am the inventor of the above-identified application, and I am qualified to design and conduct experiments related to DNA vaccines, particularly as they relate to the claimed invention. As evidence of my qualifications, attached hereto is a copy of my resume.

2. I am an employee of Aeras, the assignee of record for the above-identified application.

3. I have reviewed the subject patent application, including the claims, and the Examiner's remarks as contained in the Office Action mailed on November 3, 2004.

4. Attached here as Exhibit A is a description (4 pages of text and 1 figure) of experiments to test the ability of a composition of genes encoding an L41F mutant cholera toxin and an antigen to elicit an immune response in a mammal, as is contemplated by the claimed invention. . The experiments were carried out under my direct supervision The results showed that compositions containing DNA encoding cholera toxin L41F mutant $CtxA1_{S41Y}$ display

substantive adjuvant activity and augment both the magnitude and persistence of the serum IgG response to the HIV antigen gp120, in comparison to the control composition. Further, no adverse (toxic) affects were observed as a result of the administration of the composition.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application and any patent issuing thereon.

Date 2-3-5 Signed

David Hone

Exhibit A

Example

Evaluation of the adjuvant properties of

S63Y and S41Y derivatives of CtxA1

Experimental aim

The aim of this experiment was to evaluate the adjuvant activity of DNA vaccines that encode S63Y and S41Y derivatives of CtxA1 in plasmids designated pRc-CtxA1$_{S63Y}$ or pRc-CtxA1$_{S41Y}$.

Methods

Construction of DNA vaccines

Plasmid pRc/CMV (Genebank accession E14286) was purchased from InVitrogen Corporation (San Diego, CA). DNA vaccine pOGL1 is described elsewhere (4).

DNA vaccines encoding S63Y and S41Y derivatives of CtxA1, designated pRc-CtxA1$_{S63Y}$ or pRc-CtxA1$_{S41Y}$, were constructed using the QuikChange® Site-Directed Mutagenesis Kit (Catalog #200518, Stratagene) according to the manufacturer's directions. The site-directed mutagenesis process entailed whole-plasmid PCR using CtxA1 DNA vaccine, pRc/CMV-CTA (2), as template. The resultant PCR-generated plasmid was digested with *DpnI* to remove the template DNA and the digested DNA was introduced into *E. coli* Stable2® by chemical transformation. The transformed bacilli were cultured on tryptic soy agar (Difco, Detroit MI) supplemented with 100-μg/ml ampicillin at 30°C for 16 hr.

Isolated colonies were selected and grown overnight in 3 ml of LB medium supplemented with 100 μg/ml ampicillin. DNA was extracted from overnight liquid

cultures using a Qiagen mini plasmid DNA preparation kit (Cat No Q7106). Plasmid PCR using primers specific for $ctxA1_{S63Y}$ or $ctxA1_{S41Y}$, and agarose gel electrophoresis were conducted to screen for an appropriate derivative; clones that tested positive for were stored at –80°C and used as the source DNA for the vaccination studies below.

Animal study methods

Specific-pathogen free, 6-8 week old female BALB/cJ mice, purchased from The Jackson Laboratory (Bar Harbor, ME), were maintained in a specific-pathogen free, micro-isolator environment, and allowed to drink and eat *ad lib*. Groups of 5 mice were vaccinated intramuscularly with admixtures containing 30 μg each of endotoxin-free (<0.5 EU per mg of DNA) pOGL1 + pRc/CMV, pOGL1 + pRc-$CtxA1_{S63Y}$ or pRc-$CtxA1_{S41Y}$ DNA suspended in normal saline (0.85% (w/v) NaCl), as described (5).

Humoral responses to gp120

Solid-phase ELISA was used to determine gp120-specific Ab titers in the sera. Briefly, 96 well microtiter plates (Nunc, Rochester, NY) were coated with 100 μl of 3 μg/ml of HIV-1_{MN} gp120 (ViroStat, Portland, Oregon) in PBS at 4°C overnight. Plates were washed three times with TBS then blocked with blotto (5% wt/vol nonfat dried milk in TBS) at room temperature for 1 hour. Serially diluted sera were added to the wells and incubated at room temperature for 2 hours then washed three times with TBS. Alkaline phosphatase-conjugated goat anti-mouse IgG (Accurate Chemical, Newington NH cat# SBA103004) diluted 1/2000 in blotto + 5% lamb serum was added and incubated at room temperature for 1 hour. The plates were washed three times with TBS before adding substrate (Gibco BRL, Carlsbad CA cat# 19589-019) and incubating for 17 minutes.

Amplifier was then added (Gibco BRL cat# 19589-019) and incubated for an additional 15 minutes. Absorbance was read at 495 nm using a Victor 1420 Multilabel Counter (EG&G Wallac, Turku, Finland).

End-point titers were calculated by plotting serum dilution *VS* absorbance and taking the value where the best-fit line intersected than the mean of the negative control row plus three standard deviations.

Results

To assess the adjuvant activity of the S63Y and S41Y derivatives of CtxA1 plasmids pRc-CtxA1$_{S63Y}$ or pRc-CtxA1$_{S41Y}$ were constructed by site-directed mutagenesis, as described above. These constructs were determined to be enzymatically inactive by assaying transfected cells for intracellular cAMP levels by ELISA (data not shown). For the immunization studies, groups of 5 mice were immunized intramuscularly on weeks 0, 2, and 16 with admixtures containing 30 μg each of endotoxin-free (<0.5 EU per mg of DNA) pOGL1 + pRc/CMV, pOGL1 + pRc-CtxA1$_{S63Y}$ or pRc-CtxA1$_{S41Y}$ DNA suspended in normal saline (0.85% (w/v) NaCl), as described (5). The mice were bled monthly over an 11-month period post-priming. Sera collected before and after vaccination were used to measure the serum IgG responses to gp120 by ELISA (1, 3).

As shown in figure 1, the serum ELISAs demonstrated that the DNA vaccines encoding CtxA1$_{S63Y}$ or CtxA1$_{S41Y}$ both display substantive adjuvant activity and augment both the titer and duration of the serum IgG response to gp120. The gp120$_{MN}$ DNA vaccine, pOGL1 admixed with the control vector pRc/CMV elicited serum IgG to gp120 that increased to a maximum titer of approximately 1:3,00 by 3 months after the initial

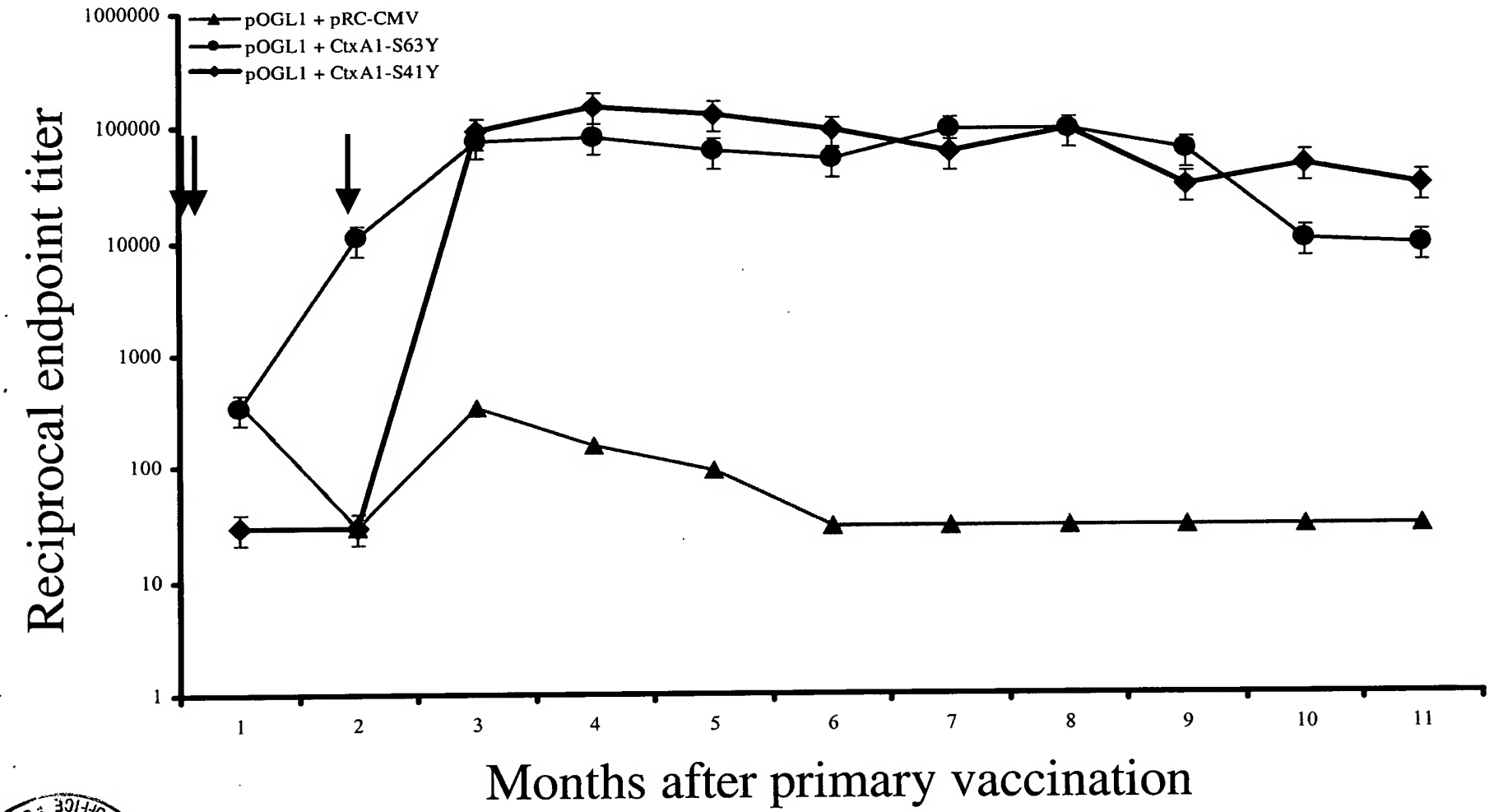
vaccination and waned thereafter. By contrast, immunization with either of the two formulations that included the DNA vaccines encoding the mutant CtxA1 dramatically enhanced both the magnitude and persistence of the anti-$gp120_{MN}$ antibody response. Thus, we conclude that DNA vaccines encoding $CtxA1_{S63Y}$ and $CtxA1_{S41Y}$ are capable of imparting potent adjuvant activity. It should be noted that at no time after the immunizations were any adverse affects observed at the immunization sites. This indicates that the mutant adjuvants were well tolerated; however, histologic analysis will be necessary to confirm this conclusion.

References

1. Abacioglu, Y. H., T. R. Fouts, J. D. Laman, E. Claassen, S. H. Pincus, J. P. Moore, C. A. Roby, R. Kamin-Lewis, and G. K. Lewis. 1994. Epitope mapping and topology of baculovirus-expressed HIV-1 gp160 determined with a panel of murine monoclonal antibodies. AIDS Res. Hum. Retrovir. 10:371-381.
2. Bagley, K. C., M. T. Shata, D. Y. Onyabe, A. L. DeVico, T. R. Fouts, G. K. Lewis, and D. M. Hone. 2003. Immunogenicity of DNA vaccines that direct the coincident expression of the 120 kDa glycoprotein of human immunodeficiency virus and the catalytic domain of cholera toxin. Vaccine 21:3335-41.
3. Pincus, S. H., K. G. Messer, D. H. Schwartz, G. K. Lewis, B. S. Graham, W. A. Blattner, and G. Fisher. 1993. Differences in the antibody response to human immunodeficiency virus-1 envelope glycoprotein (gp160) in infected laboratory workers and vaccinees. J. Clin. Invest. 91:1987-1996.
4. Shata, M. T., and D. M. Hone. 2001. Vaccination of mice with a *Shigella*-gp120 DNA vaccine vector induces HIV-1 gp120-specific $CD8^+$ T cells and antiviral protective immunity. J. Virol. 75:9665-9670.
5. Wolff, J. A., R. W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani, and P. L. Felgner. 1990. Direct gene transfer into mouse muscle in vivo. Science 247:1465-1468.

Exhibit A

Adjuvant properties of S63Y and S41Y derivatives of CtxA1



BIOGRAPHICAL SKETCH

NAME:- David M. Hone **POSITION TITLE:-** Chief Scientific Officer

Education and Training

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
University of Adelaide, Australia	B.Sc.(Hons.)	1984	Genetics of lipopolysaccharide biosynthesis
University of Adelaide, Australia	Ph.D.	1984-1988	*Salmonella* vaccines and vaccine vectors
University of Maryland, Baltimore, USA	Postdoc	1988-1990	*Salmonella* and *Shigella* vectors

Professional Experience

1990-1995 Assistant Professor, Division of Geographic Medicine, Department of Medicine, University of Maryland at Baltimore.

1992-1995 Chief, Salmonella Vaccine Vector Unit, Center for Vaccine Development, University of Maryland at Baltimore.

1996 Assistant Professor, Institute of Human Virology, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore.

1997-2003 Associate Professor, Institute of Human Virology, UMBI, Baltimore.

1999-2003 Acting Director, Division of Vaccine Research, Institute of Human Virology, UMBI, Baltimore.

2003-Present Chief Scientific Officer, Aeras Global TB Vaccine Foundation, Rockville, MD

Editorial Assignments

1995-1997 Editorial Board, Infection and Immunity.

1990-present Vaccine, Proceeding of the National Academy of Sciences USA, Journal of Infectious Diseases, New England Journal of Medicine, Journal of Cellular and Molecular Biology.

Grant Review Committees

1993 Technical Review Committee, NIH-NIAID-DAIDS RFP 94-05. Collaborative Mucosal Immunology Groups for AIDS Vaccines.

1993-1996 Bacterial and Mycology Small Business Innovative Research Study Section, National Institute of Allergy and Infectious Disease.

1994-1999 Reviewer, Steering Committee on Diarrhoeal Disease Vaccines, WHO Global Program for Vaccines and Immunization. WHO, Geneva, Switzerland.

1999 Technical Review Committee, NIAID-DAIDS RFP 00-010. HIV Vaccine Design and Development Teams.

2001 Technical Review Committee, NIAID-DAIDS RFP 02-06. HIV Vaccine Design and Development Teams.

2005 Technical Review Committee, NIAID-ZRG1 VACC (01), HIV Vaccine Study Section.

Selected Publications (from a total of 62 publications and patents)

1. Onyabe D.Y., Fouts T.R., and D.M. Hone. Characterization of humoral immune responses to recombinant HIV-1 gp120 genetically linked to human CD4 and a CD4 mimetic (*Submitted*).
2. Wang X., Kochetkova I., Haddad A., Hoyt T., Hone D.M., and D.W. Pascual. 2005. Trangene vaccination using *Ulex europaeus* agglutinin I for targeted mucosal immunization against HIV-1 envelope. (*Submitted*)
3. Ahearn A.J., Kalyanaraman V.S., Popovic M., and D.M. Hone. 2005. Tat-stimulated dendritic cell supernatants promote $CD8^+$ T cell activation but not cytolytic effector functions. (*Submitted*)
4. Sadoff J.C. and D.M. Hone. 2005. The role of Go No-Go decisions in TB vaccine development. *In*: Microbes and Infection: Focus on rational vaccine development against tuberculosis. S.E. Kauffman (Ed.). (*Submitted*)
5. Wang X., Hone D.M., Haddad A., Shata M.T., and D.W. Pascual. 2003. M Cell Vaccination for Pulmonary CTL. *J. Immunol.* 171:4717-4725.

6. Fouts T.R., DeVico A.L., Onyabe D.Y., Shata M.T., Bagley K.C., Lewis G.K. and D.M. Hone 2003. Progress toward the development of a bacterial vaccine vector that induces high-titer long-lived broadly neutralizing antibodies against HIV-1: *FEMS Immunol. Med. Microbiol.* **37**:129-134.
7. Bagley K.C., Shata M.T., Onyabe D.Y., DeVico A.L., Fouts T.R., Lewis G.K., and D.M. Hone. 2003. Immunogenicity of DNA vaccines that direct the coincident expression of the 120 kDa glycoprotein of HIV-1 gp120 and the catalytic domain of cholera toxin. *Vaccine*, **21**:3335-3341.
8. Agwale S.M., Shata M.T., Reitz M.S. Jr., Kalyanaraman V.S., Gallo R.C., Popovic M. and D.M. Hone. 2002. A Tat subunit vaccine confers protective immunity against the immune-modulating activity of the human immunodeficiency virus-1 Tat protein in mice. *Proc. Natl. Acad. Sci.*, 99:10037-1041.
9. Hone D.M., DeVico A.L., Fouts T.R., Onyabe D.Y., Agwale S.M., Wambebe C.O., Blattner W.A., Gallo R.C. and G.K. Lewis 2002. Development of vaccination strategies that elicit broadly neutralizing antibodies against HIV-1 in both the mucosal and systemic immune compartments. *J. Human Virol.*, 5:17-23.
10. Shata M.T., Lewis G.K., and D.M. Hone. 2001. Intragastric vaccination with a *Salmonella* Env DNA vaccine vector elicits mucosal HIV-1 envelope-specific $CD8^+$ T cells. *Vaccine*, 20:623-629.
11. Shata M.T. and D.M. Hone. 2001. Vaccination of mice with a *Shigella*-gp120 DNA vaccine vector induces of HIV-1 gp120-specific $CD8^+$ T cells and anti-viral immunity. *J. Virol.*, 75:9665-9670.
12. Shata M.T., Stevceva L., Lewis G.K., and D.M. Hone. 2000. Recent advances with recombinant bacterial vaccine vectors. *Molec. Med. Today*, 6: 66-70.
13. Fouts T.R., Tuskan R., Godfrey K., Reitz M., Hone D.M., Lewis G.K., and A.L. DeVico. 2000. Expression and characterization of a single-chain polypeptide analogue of the human immunodeficiency virus type 1 gp120-CD4 receptor complex. *J. Virol.*, 74:11427-11436.
14. Hone D.M., Shata M.T., Pascual D.W., and G.K. Lewis. 1999. Mucosal vaccination with *Salmonella* vaccine vectors. *In*: Intracellular bacterial vaccine vectors: immunology, cell biology and genetics. J. Wiley and Sons. New York, NY. Pp 171-222.
15. Pascual D.W., Hone D.M., Hall S., van Ginkel F.W., Yamamoto M., Walters N., Fujihashi K., Powell R.J., Wu S., VanCott J.L., Kiyono H., and McGhee J.R. 1999. Expression of recombinant enterotoxigenic *Escherichia coli* colonization factor antigen I by *Salmonella* elicits a biphasic T helper cell response. *Infect. Immun.*, 67: 6249-6256.
16. VanCott J.L., Chatfield S.N., Roberts M., Hone D.M., Hohman E., Pascual D.W., Yamamoto M., Yamamoto S., and J.R. McGhee. 1998. Regulation of bacterial attenuation and toxicity by modification of virulence genes. *Nature Med.*, 11: 1247-1252.
17. Hone D.M., Powell J., Crowley R., Maneval D., and G.K. Lewis. 1998. Lipopolysaccharide from an *Escherichia coli htrB msbB* mutant induces high levels of MIP-1α and MIP-1β secretion without inducing TNFα and IL-1β. *J. Human Virol.*, 1: 251-256.
18. Wu S., Pascual D.W., Lewis G.K., and D.M. Hone. 1997. Induction of mucosal and systemic responses against human immunodeficiency virus type-1 gp120 in mice after oral immunization with a single dose of a *Salmonella*-HIV vector. *AIDS Res. Hum. Retrovirus.*, 13: 1187-1194.
19. Powell R.J., Lewis G.K., and D.M. Hone. 1996. Introduction of eukaryotic expression cassettes into animal cells using a bacterial vector delivery system. *In: Vaccines 96., "Molecular approaches to the control of infectious disease"*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.. Pp183-187.
20. Fouts T.R., Lewis G.K., and D.M. Hone. 1995. Construction and characterization of a *Salmonella*-based human immunodeficiency virus type 1 vector vaccine. *Vaccine*, 13: 561-569.
21. Noriega F., Hone D.M., and M.M. Levine. 1994. Construction and characterization of attenuated $\Delta aroA$, $\Delta virG$ *Shigella flexneri* 2a strain CVD 1203, a prototype oral vaccine. *Infect. Immun.*, 62: 5168-5172.
22. Hone D.M., Harris A.M., and M.M. Levine. 1994. Construction and characterization of isogenic O-antigen variants of *Salmonella typhi*. *Molec. Microbiol.*, 13: 525-530.
23. Hone D.M., Tacket C.O., Harris A., Kay B., Losonsky J., and M.M. Levine. 1992. Evaluation in volunteers of a candidate live oral attenuated *Salmonella typhi* vector. *J. Clin. Invest.*, 90:412-420.
24. Hone D.M., Harris A., Chatfield S., Dougan G., and M.M. Levine. 1991. Construction of genetically defined double *aro* mutants of *Salmonella typhi*. *Vaccine*, 9:810-816.
25. Aggarwal A., Kumar S., Jaffe R., Hone D.M., Gross M., and J. Sadoff. 1990. Oral *Salmonella*: Malaria circumsporozoite recombinants induce specific $CD8^+$ cytotoxic T cells. *J. Exp. Med.*, 172:1083-1090.
26. Hone D.M., Attridge S.R., Forrest B., Morona, R., Daniels, D., LaBrooy J.T., Bartholomeusz R.C., Shearman D.J., and J. Hackett, 1988. A *galE via* (Vi Antigen-negative) mutant of *Salmonella typhi* Ty2 retains virulence in humans. *Infect. Immun.*, 56:1326-1333.